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The polycation conducting polymer, oxidized polypyrrole, possesses the ability to form complexes with DNA. Our previously proposed diffusion limited binding model for double helical DNA was also found to be applicable to single stranded DNA in this study. However, single stranded DNA was found to bind PPy at a higher level than double helical DNA. An investigation of electropolymerized PPy film morphology using SEM and TEM techniques revealed two distinctly differing surface morphologies for the Platinum (Pt) electrode face (smooth) and polymeric growth face (rough). The DNA uptake levels were found to be consistently different on either surface. Double stranded DNA penetration into the surface increased with extended time periods of exposure while a similar phenomenon, but to a lower extent, was observed for single stranded DNA.

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COMPARISON OF SINGLE AND DOUBLE STRANDED DNA BINDING TO POLYPYRROLE.

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ABSTRACT

The polycation conducting polymer, oxidized polypyrrole (PPy), possesses the ability to form complexes with DNA. Our previously proposed diffusion limited binding model for double helical DNA was also found to be applicable to single stranded DNA in this study. Single stranded DNA was found to bind PPy at a nearly identical level to that of double helical DNA. An investigation of electropolymerized PPy film morphology using SEM revealed two distinctly differing surface morphologies for the Platinum (Pt) electrode face (smooth) and polymeric growth face (rough). The DNA uptake levels were found to be consistently different on either surface, being higher on the rough surface. DNA penetrated into the disk interior with increasing time periods of exposure while a similar phenomenon but to a lesser extent was observed for single stranded DNA.

INTRODUCTION

The oxidized form of polypyrrole (PPy) is electrically conducting with conductivity in the range of 10^{-3} - 10^{2} S cm^{-1} [1,2]. Conduction in PPy is due to the mobility of positive charged defect structures in the highly conjugated polymer backbone [3,4]. The cations form donor - acceptor complexes with electrolyte ions during the synthesis of the conducting polymer. Past studies performed by our group have shown that polyanionic DNA binds PPy, presumably by replacing the dopant counterions upon binding. We have demonstrated that the uptake kinetics of double helical DNA follows a classical diffusion limited adsorption model with no significant activation energy to binding [5 -7].

Observation of the surface of electropolymerized PPy disks using scanning

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electron microscopy (SEM) has revealed two distinct morphologies, a rough surface (polymer growth face) characterized by the presence of grooves, channels and craters, and a smooth surface (electrode face) devoid of these structures. Since DNA binding routes could involve diffusion or capillary uptake into internal channels as well as surface adsorption in the PPy disk we undertook the following studies. A comparative study of the binding properties of single and double stranded DNA to polypyrrole was undertaken by counting decay events from both sides of PPy disks to determine time dependent DNA penetration into the disk interior. The DNA binding and matrix penetration phenomena may find uses for PPy as a new material in biotechnology applications. The optoelectronic properties of conducting polymers may find uses in signal transduction in biosensors where nucleic acids form the molecular recognition system utilizing molecular hybridization.

EXPERIMENTAL METHODS

The electrochemical polymerizations were performed as previously described [7]. After 4 hours of reaction time a film of 100 - 130 μm thickness was deposited on the Pt electrode. The free standing film was peeled off with a razor blade and tweezers, followed by soaking in acetonitrile for 24 hours and drying at 25° C for 12 hours. The film was cut into circular disks 0.60 cm in diameter and stored in the dark.

Native pBR 322 DNA (New England Biolabs) was linearized by restriction cleavage with EcoRI (New England Biolabs). The linearized DNA was then ^{35}S radiolabeled using a 3' end labeling kit NEK 009Z (New England Nuclear) achieving a specific activity of 5 X 10^4 cpm/pmol. The radiolabeled DNA was dissolved in 500 μl of TE buffer (1 mM EDTA and 10 mM Tris, pH 8) and was stored at - 20° C. Samples containing 160 nanograms of ^{35}S DNA in a 200 μl droplet in 1X TE buffer were placed on a polypropylene tray. The tray was placed in a petri dish which contained a reservoir of 1X TE buffer to minimize sample droplet evaporation. The disk shaped PPy substrate was then placed upon the DNA solution droplets for varying lengths of time at 37° C. Following exposure to the DNA droplet, the substrate was treated to three 10 minute washes in 1X TE buffer. For single stranded DNA binding 160 nanograms of DNA were aliquoted out of the stock solution. This was heated in a water bath at 95° C for 10 minutes and immersed into ice cold 1X TE buffer to a final volume of 200 μl.

Radioactivity on disks was detected by both scintillation counting of total radioactivity and using a high voltage proportional counter for counting from rough and smooth disk faces. Samples were counted over 200 minute periods. A substrate disk, treated identically without being exposed to the radiolabeled DNA was used as a control. All the raw sample counts were corrected for

background (control value) radiation levels. Scanning electron microscopy was performed as previously described [6].

RESULTS AND DISCUSSION

The low resolution scanning electron microscope (SEM) images in figure 1 reveal differences in the texture of the rough (R) and smooth (S) surfaces and cross-sectional views of the electropolymerized PPy film used in DNA binding experiments.

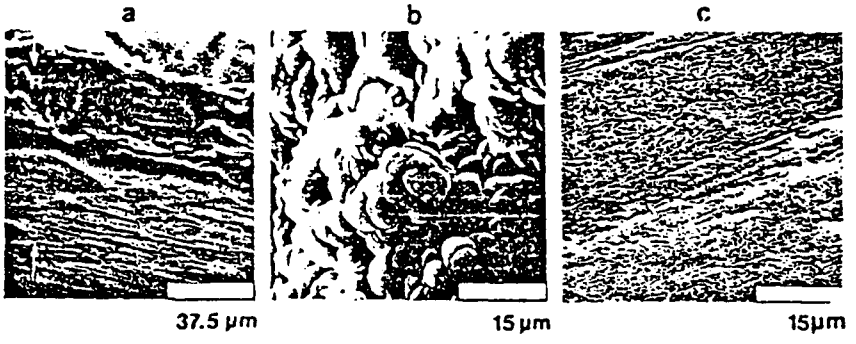

Fig.1. SEM images of PPy (a) Cross-sectional end view, (b) surface morphology of the rough surface, (c) surface morphology of the smooth surface.

The conductivity measurements of the two sides being identical, the only difference observed relevant to DNA binding is the difference in the surface morphology. The rough side of the film (polymer growth face) shows a regular array of craters and bumps whereas the smooth side (Pt electrode face) is devoid of any such structures. In fact preliminary studies (data not shown) have shown that DNA binding is higher on the rough surface than on the smooth surface which would agree with our SEM observations of the apparent lower surface area of the smooth side relative to the rough side.

Rough face binding kinetics experiments of single and double stranded DNA shown in figure 2 are in agreement with the previously suggested diffusion limited binding model which is expressed in equation 1.

$$n = 2C(Dt/\pi)^{1/2} \qquad (1)$$

where n is the number of molecules binding per unit area, C is the bulk solution DNA concentration, D is the diffusion coefficient and t is the time.

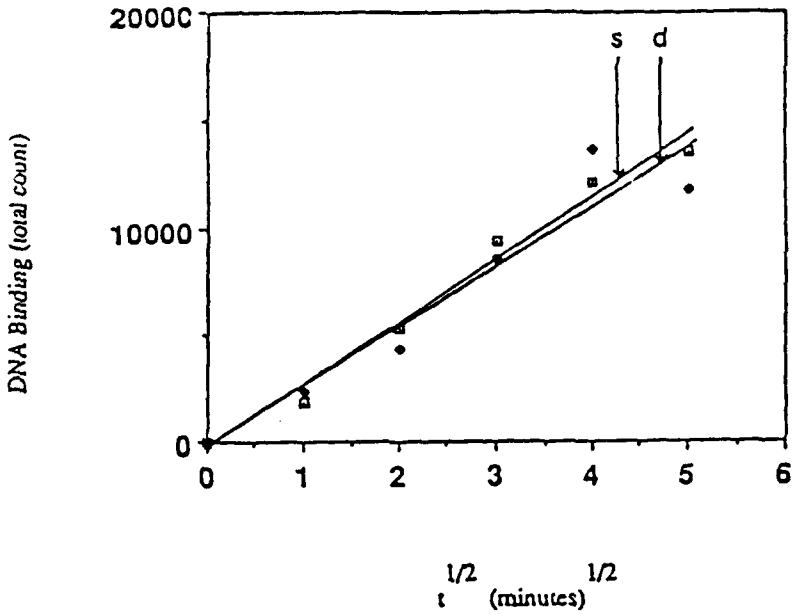

Fig. 2. DNA binding kinetics onto the rough surface of PPy: (▫) single stranded DNA (s); (•) double stranded DNA (d). Best fit linear regressions to these data are shown.

It can be seen that for the initial stages of binding, a $t^{1/2}$ dependence is observed. Our studies seem to suggest that single stranded DNA binding to PPy takes place similarly to that of the double stranded DNA. We measured the R / S radioactivity ratio (the ratio of radioactivity detected on the rough surface to that detected on the smooth surface) for rough side uptake of both the single and double stranded DNA as shown in figure 3. There was a progressive decrease in the R / S ratio for both DNAs indicating that at longer exposure times each DNA was penetrating the disk and moving toward the smooth side. The higher level of R / S for the single stranded DNA might be explained by its decreased mobility once bound to PPy. The exposed bases of single stranded DNA may have the capability to bind PPy by hydrogen bonding or even through base stacking interactions with the PPy backbone ring system. These bonding opportunities are absent in double stranded DNA and may be responsible for its higher internal mobility and consequently lower R / S ratio. The quantitative levels of R / S ratios agree with a simple experiment indicating that the half attenuation thickness for PPy measuring S^{35} disintegration was about 40 microns. For 120 micron disks the ratio should be about 8:1.

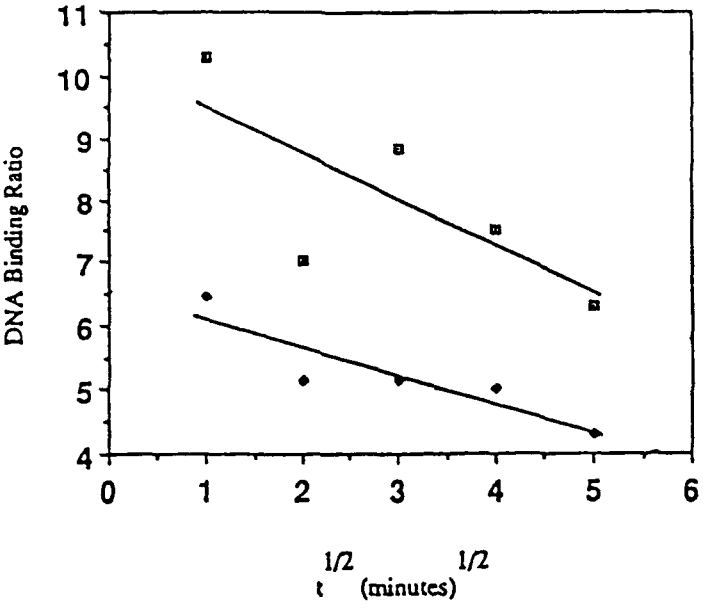

Fig. 3. Ratio of R / S surface radioactivity following rough surface DNA uptake on PPy : (▫) single stranded ; (•) double stranded DNA uptake. Best fit linear regression to these data are shown.

These binding results suggested we probe the surface of the PPy films at very high resolution using TEM techniques. We have undertaken such studies and preliminary TEM images of the rough surface (data not shown) shows the presence of an abundance of grooves or channels [9]. The dimensions of these channels support our observations that the DNA could easily penetrate into these channels and move towards the interior. The smooth surface is almost devoid of such channels and has the appearance of very regular and dense chain packing. We have not yet determined whether DNA penetrates this surface. These observations are thus in agreement with our DNA binding experiments.

CONCLUSIONS

We have demonstrated that the diffusion limited binding model proposed for double stranded DNA applies to single stranded DNA as well. The penetration of DNA from the rough surface into the interior of the disk has been observed. This phenomenon is in agreement with SEM and high resolution TEM images showing channels and grooves on the rough surface. The DNA binding and matrix penetration phenomena may find uses for PPy as a new material in biotechnology applications.

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REFERENCES

(1) J. Roncali, A. Yassar and F. Garnier, Synthetic Metals, 28 C 275 (1989).
(2) S. Hotta, Synthetic Metals, 22, 103 (1987); 26, (1988).
(3) A. F. Diaz and J. C. Lacroix, New J. Chem, 12, 171 (1988).
(4) B. D. Malhotra, N. Kumar and S. Chandra, Prog. Poly. Sci., 12 179 (1988).
(5) D. S. Minehan, K. A. Marx and S. K. Tripathy, Macromolecules in Press.
(6) J. O. Lim, D.S. Minehan, M. Kamath, K. A. Marx and S. K. Tripathy MRS Proceedings 255 (1992).
(7) D. S. Minehan, K. A. Marx and S. K. Tripathy, Proc. Amer. Chem. Soc. Div. Polymeric Materials: Science & Eng. 64, 341-342
(8) J. S. Vrentas and J. L. Duda, "Diffusion", in Encyclopedia of Polymer Science and Engineering, Vol. 5, eds. H.F. Mark et al. (John Wiley Inc, New York 1986), pp 462-507.
(9) K. A. Marx, G. C. Ruben, Rajiv Pande, J. O. Lim, S. K. Tripathy and D. L. Kaplan, manuscript in preparation.